A close examination of the time course in *B mori* reveals that, prior to the decrease the pH peaks (Fig 1) The mechanism mediating this rise is unknown, but may be a non-specific stress response to the force-feed procedure.

Luminal K^+ activity is largely unaffected by Bt intoxication, but K^+ activity in the haemolymph increases by more than 60%, abolishing even the small chemical gradient that existed under equilibrium conditions (Table 1). The source of the additional haemolymph K^+ is uncertain, but may well be the midgut lumen itself. As the transmidgut PD decreases and luminal contents become acidified, it is possible that protein-bound K^+ is released[8] and driven toward the haemolymph side until the activities in both compartments become equal.

Irreversible destruction of the midgut epithelium by Bt δ-endotoxins is caused by a combination of cell alkalization[10] and colloid osmotic lysis.[11] Most insects susceptible to Bt, however, do not succumb directly to the endotoxin itself, but to the septicemia that follows, either from germinating Bt spores in the gut, if challenged with spore-crystal mixtures, or from other opportunistic pathogens that may be present in the gut.

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Molecular detection of insecticide resistant alleles

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Abstract: The summary discusses techniques used to investigate mechanisms of resistance in the Colorado potato beetle (*Leptinotarsa decemlineata*) to several classes of pesticides.

Keywords: Colorado potato beetle; resistance; mutation; single-stranded polymorphism; PCR amplification of specific alleles; DNA mini-sequencing

The Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* (Say)) is a world-wide pest of potatoes and is resistant to all major classes of insecticides. Detailed biochemical and pharmacological studies have established a variety of resistance mechanisms; including altered acetylcholinesterases (organophosphates and carbamates),[1] insensitive ion channels (DDT and pyrethroids),[2] sequestration proteins associated with the hemolymph (multiple insecticides)[3] and induced oxidative metabolism (abamectin).[4] We have devised a number of molecular-based diagnostic procedures to detect mutations and over-expressed proteins that result in resistance, including single-stranded conformational polymorphism (SSCP), PCR amplification of specific alleles (PASA) and mini-sequencing of DNA coupled with immunochemical detection.

A point mutation in the acetylcholinesterase (AChE) gene associated with azinphos-methyl resistance

We have cloned and sequenced a cDNA encoding the AChE of azinphosmethyl-susceptible (SS) strain of CPB.[1] The deduced amino acid sequence consisted of 29 residues for the putative signal peptide and 600 residues for the mature protein. A point mutation (A→G, nt location 980) that resulted in a Ser (AGT)/Gly (GGT) amino acid change (designated [S291G])[5] was identified in the azinphos-methyl-resistant strain of CPB (AZ-R). The A→G mutation was found in all AChE cDNA sequences amplified by PCR from enzymatically authenticated AZ-R beetles but was never found in AChE cDNA sequences from SS beetles.[5] The Ser/Gly change, however, does not occur within either the esteratic subsite or the peripheral anionic site of AChE. This amino acid residue corresponds to Val 238 of the *Torpedo* AChE and represents the first residue to form the α-helix, $\alpha\text{-E}'_1$. The predicted secondary structure of the mutation-containing region of the AZ-R AChE indicates that the transition from the turn sequence to the α-helix sequence occurs sooner in the sequence when Ser is replaced by Gly. Thus, the Ser/Gly change is

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(*Received 16 July 1998; accepted 5 January 1999*)

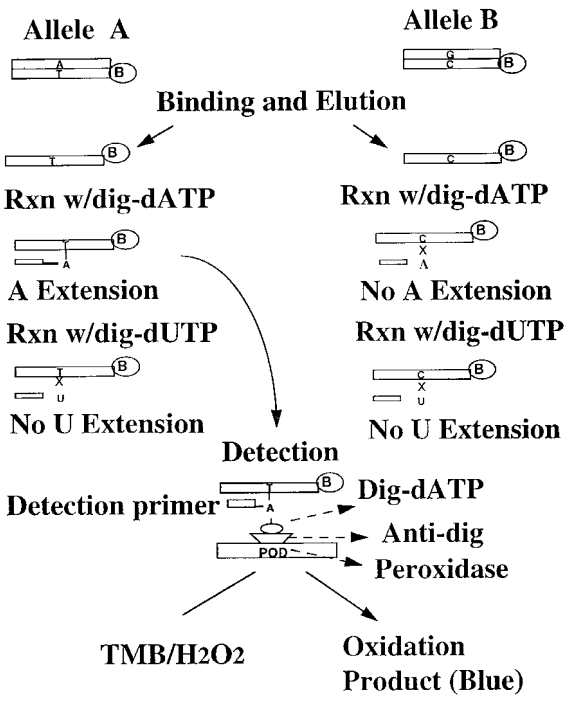

Figure 1. Schematic of mini-sequencing reaction.

consistent with a destabilization in the $\alpha E'_1$-helical structure leading to an altered secondary structure. Conformational changes in the AChE due to the helix deformation are expected to impinge upon both the catalytic and peripheral binding sites due to the location of the Ser/Gly change.

A point mutation in the para-type sodium channel α-subunit zgene associated with knockdown resistance (kdr-like) to pyrethroids and DDT

In collaboration with Professor D M Soderlund and Dr S H Lee at Cornell University, Geneva, we have identified a C→T mutation in the S6 transmembrane spanning unit of domain II (IIS6) of the *para* homologous sodium channel α-subunit gene of the permethrin-resistant (PE-R) strain of CPB. This point mutation results in an amino acid change from a leucine (CTT) to a phenylalanine (TTT) (designation [L1014F]). CPB larvae (both very susceptible, VS, and PE-R strains) were pre-treated twice with α-naphthyl caproate, prior to the treatment with permethrin and the assessment of knockdown, in order to saturate the permethrin carboxylesterase. A 544 bp cDNA fragment that spanned the corresponding [L1014F] mutation was generated by PCR. The forward primer was designed against the anti-sense strand in the extracellular loop between the S3 to S4 transmembrane units of domain II and the reverse primer was designed against the sense strand in the intracellular loop between domains II and III. Amplified DNA was sub-cloned by TA cloning for automatic sequencing. We have now validated that the C→T mutation resulting in the leucine (L) → phenylalanine (F) substitution ([L1014F]) only occurs in beetles from the PE-R strain and is never found in beetles in the VS strain.

Using these two point mutations, we have established procedures, based on three DNA diagnostic techniques (SSCP, mini-sequencing, cPASA), for the efficient and rapid detection of the susceptible (AGT) and resistant (GGT) alleles associated with azinphos-methyl resistance and the susceptible (CTT) and resistant (TTT) alleles associated with permethrin resistance. Initial detection of resistant alleles is accomplished by SSCP. Genomic DNA samples were PCR amplified as described by Zhang *et al.*[6] The homologous primers amplified a 163 bp sequence that contained the [S291G] mutation site associated with azinphos-methyl resistance and a 349 bp sequence that contained the [L1014F] mutation site associated with the *kdr*-like permethrin resistance. Genomic DNA polymorphisms from the AZ-R versus SS and the PE-R versus VS strains were detected by silver staining following PAGE.

SSCP results were validated by direct sequencing using the mini-sequencing reaction (Fig 1). In this format, only the leucine (CTT) to phenylalanine (TTT) mutation associated with permethrin resistance can be assessed directly by an increase in optical density (OD) (Table 1). In the case of azinphos-methyl resistance, only the susceptible (AGT) allele

Table 1. Mini-sequencing results (as measured by optical density, OD_{450}) obtained from digoxigenin-labeled dATP or dUTP reactions with amplified genomic DNA templates containing the [L1014F] point mutation site of the *para* sodium channel gene from VS and PE-R strains of Colorado potato beetle

CPB	OD_{dATP}	$OD_{dATP} - OD_{ddH2O}$	*Mean (±SD)*	OD_{dUTP}	$OD_{dUTP} - OD_{ddH2O}$	*Mean (±SD)*
VS1	0.117	0.035		0.122	0.062	
VS2	0.097	0.015		0.086	0.026	
VS3	0.101	0.019		0.136	0.076	
VS4	0.120	0.038	0.027 (±0.011)	0.129	0.069	0.058 (±0.022)
PE-R1	0.382	0.300		0.065	0.005	
PE-R2	0.373	0.291		0.071	0.011	
PE-R3	0.402	0.320	0.304 (±0.015)[b]	0.106	0.046	0.021 (±0.022)
H_2O[a]	0.082			0.060		

[a] Double-distilled water was added instead of PE-R genomic DNA template as control.

[b] Mean value was significantly different from VS mean value ($P < 0.001$).

Table 2. Mini-sequencing results (as measured by optical density, OD_{450}) obtained from digoxigenin-labeled dATP or dUTP reactions with amplified genomic DNA templates containing the [S291G] point mutation site of the AChE gene from SS and AZ-R strains of Colorado potato beetle

CPB	OD_{dATP}	$OD_{dATP} - OD_{ddH2O}$	Mean (±SD)	OD_{dUTP}	$OD_{dUTP} - OD_{ddH2O}$	Mean (±SD)
SS1	0.407	0.304		0.174	0.115	
SS2	0.434	0.331		0.104	0.045	
SS3	0.49	0.387		0.137	0.078	
SS4	0.434	0.331	0.338 (±0.035)	0.125	0.066	0.076 (±0.029)
AZ-R1	0.155	0.052		0.127	0.068	
AZ-R2	0.116	0.013		0.112	0.053	
AZ-R3	0.123	0.02	0.028 (±0.021)[b]	0.148	0.089	0.070 (±0.018)
ddH_2O[a]	0.103			0.059		
SS5	0.39	0.292		0.125	0.076	
SS6	0.369	0.271		0.142	0.093	
SS7	0.402	0.304		0.156	0.107	
SS8	0.425	0.327	0.301 (±0.023)	0.153	0.104	0.095 (±0.014)
AZ-R4	0.14	0.042		0.101	0.052	
AZ-R5	0.148	0.05		0.182	0.133	
AZ-R6	0.115	0.017	0.036 (±0.017)[b]	0.154	0.105	0.097 (±0.041)
ddH_2O[a]	0.098			0.049		

[a] Double-distilled water was added instead of AZ-R genomic DNA template as control.
[b] Mean value was significantly different from SS mean value ($P < 0.001$).

results in an increased OD (Table 2). However, the presence of the resistant GGT allele can be directly determined using cPASA as previously described by Zhu and Clark.[7]

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Ligands of the nicotinic acetylcholine receptor as insecticides

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Abstract: Insect nicotinic acetyl receptors (nAChR) are targets of growing importance and, since the early 1990s, the number of such highly effective insecticides as imidacloprid and spinosyn has grown. Several natural compounds, eg dihydro-β-erythroidine, methyl caconitine and paraherquamide, showing high affinity to the same receptor, were considerably less active as insecticides, most likely because of their antagonistic action. Our observations on aphids after ingestion of the antagonistic compound dihydro-β-erythroidine revealed anti-feedant-like properties. As a consequence, the symptomology of poisoning was totally different between agonists and antagonists of the nAChR. Electrophysiological (whole-cell voltage clamp) measurements in isolated housefly neurones revealed that agonism seems to be a prerequisite for insecticidal activity. Furthermore, we were able to demonstrate the existence of two different subtypes of the nAChR in isolated locust neurones with different pharmacology and ion-channel properties.

Keywords: Imidacloprid; neonicotinoids; chloronicotinyls; dihydro-β-erythroidine; methyl caconitine; paraherquamide; nicotinic acetylcholine receptor; *Myzus persicae*; electrophysiology; antifeedant; *Locusta migratoria*; *Musca domestica*

Background

Only ten years ago insecticides acting on the nicotinic acetylcholine receptor (nAChR) were of minor economic importance (<2% of the total insecticide market in 1991) and registered compounds included cartap (1964), thiocyclam (1977) and bensultap (1968) which were metabolised to nereistoxin, a naturally occurring toxin described in the marine worm *Lumbriconereis heteropoda* Marenz, within the insect's body. Nicotine is one of the oldest known insecticides and the compound is still used to control some homopteran pests in greenhouses.

A totally different class of compounds which affect nAChR with considerable biological efficacy against key homopteran and coleopteran pests are the chloronicotinyls or neonicotinoids,[1] a new chemical class of insecticide introduced to the market in the early 1990s.

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(*Received 13 October 1998; accepted 5 January 1999*)